DETAILED ACTION

1. This action is in response to papers filed 6/16/2008.

2. Currently Claims 1-49 and 52-63 are pending. Claims 50-51 have been cancelled.

3. The following rejections for Claims 1-49 and 52-63 are newly applied. Response to arguments follows.

4. The office action of 9/29/2008 was mailed out as a final rejection has been withdrawn. After an interview with Shirley Recipon the final office action of 9/29/2008 has been withdrawn and the following nonfinal office action is presented below.

5. This action is Non FINAL.

Withdrawn Rejections

6. The rejection of the claims under 35 USC 112/2nd paragraph made in section 3 of the previous office action is moot based upon amendments to the claims.

7. The rejection of the claims under 35 USC 102(b) and 35 USC 103(a) made in sections 4-24 of the previous office action are moot based upon the amendments to the claims. It is noted that some of the art made of record in the previous office action has been used in the following rejections with newly applied art necessitated by amendment. Therefore the arguments presented in the reply are discussed following rejections.

Interview Summary

8. The examiner acknowledges the interview summary presented in the reply on p. 14 last paragraph.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

9. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

10. The following 35 USC 103(a) rejection are newly applied as necessitated by amendment. It is noted that the discussion of Wittwer et al. has been placed on record

in the previous office action, however, based upon amendments tot he claims, has been made into a 35 USC 103(a) rejection in view of Marras et al.

11. Claims 1-4, 6, 9-10, 26-28, 31-33, 36-44, 46-59, and 61-62 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wittwer et al. (US Patent 6,140,054 October 31, 2000) in view of Marras et al. (Nucleic acids Research 2002 Vol. 30 p. e122)

Wittwer et al. teaches a method of mutation detection using fluorescently labeled oligonucleotide hybridization probes to identify mutations and polymorphisms based on melting curve analysis of hybridization probes.

With regard to Claims 1, 10, 36, 46-49, Wittwer et al teaches contacting a sample with a first signal probe (donor) which is capable of hybridizing to at least a portion of a first target, a first quencher (acceptor) capable of hybridizing in proximity to the first signal probe) wherein the quencher has a Tm below that of the first signal probe (Column 4 lines 26-35, Column 12 lines 18-45). Wittwer et al. teaches the detection of sequence alterations at two or more loci of an amplified DNA sequence determined by melting-point analysis (Column 4 lines 20-25). Wittwer et al. teaches hybridization probes are designed to hybridize to the amplified region and span the mutation site (Column 4, lines 26-29). Wittwer et al. teaches a method using FRET oligonucleotide pair comprised of a donor and acceptor (Column 4 lines 32-35). Wittwer et al teaches a method in which multiple loci of the target nucleic acids sequence can be analyzed using sets of FRET oligonucleotide pairs in which have different melting temperatures

(e.g. a second quencher and signal) (Column 12, lines 40-45). Wittwer et al. teaches that either the donor or acceptor (signal or quencher) can pan across the mutation (Column 12, lines 18-20). Wittwer et al. teaches the melting temperature of the probe hybridized to the mutation locus has a lower melting temperature then the other probe in the FRET pair (column 12, lines 29-34). Wittwer teaches that when the acceptor probe is designed to hybridize to the mutation then it has a lower melting temperature than the donor probe.

Wittwer et al. teaches the fluorescence of the sample is monitored as a function of temperature (Column 14, lines 38-39). In Figure 1, Wittwer et al. teaches detection of fluoresce (over time as a function of melting temperature. In Figure 1, Wittwer et al. discloses rather the signal is emitted or not emitted. Therefore Wittwer et al. teaches monitoring the turning-off or turning-on of the signal depending on the melting temperature of the probes.

With regard to Claims 1, 10, 36, and 46-40, Wittwer et al. however does not directly detecting the detectable signals of the signal probes as a function of temperature, and instead teaches indirectly detecting such signals. However, as taught below Marras et al. provides the direct detection of the signal and motivation to detect.

With regard to Claim 2, Wittwer teaches a method of using and monitoring fluorescent probes (Column 7 lines 60-61).

With regard to Claim 3, Wittwer et al. teaches that multiple sets of FRET pairs can be labeled with different fluorescent resonance energy transfer pairs so that the

sets of FRET oligonucleotide pairs can be distinguished from one another based on the distinguishable emission spectra (Column 12, lines 57-61).

With regard to Claim 4, Wittwer et al. teach FRET oligonucleotide pairs having different melting temperatures for each of the FRET oligonucleotide pair are preferred (Column 13, lines 9-11).

With regard to Claims 6 and 9, Wittwer et al. teaches different sets of FRET oligonucleotide pairs can be labeled with the same fluorescent resonance transfer pair, allowing for monitoring at a single emission wavelength (Column 12 lines 49-53).

With regard to Claims 26-28 and 31-33, Wittwer et al. teaches fluorescence monitoring of the PCR reaction is used to acquire product melting curves during the PCR reaction (Column 15 lines 56-57). Wittwer et al. teaches the temperature cycles of PCR that drive amplification alternately denature the accumulating product and the fluorescently labeled hybridized probes at a high temperature, and anneal the primers and the hybridization probes to the product at a lower temperature (Column 15 lines 59-63). Therefore it is inherent in the teaching of Wittwer et al. the melting temperature is measured by as an increase in temperature and a decrease in temperature. Wittwer et al. teaches temperature cycling of 94°C to 75°C which are above the melting point of the quencher probe and below the melting point of the signal probe (Column 20 lines 25-30).

With regard to Claim 37 and 38, Wittwer et al. teaches a method in which the polynucleotide sample is an amplified product which is single stranded (Column 4, lines 20-25).

With regard to Claims 39-41, Wittwer et al. teaches multiple loci of a target nucleic acid sequence can be analyzed (column 12 lines 41-42).

With regard to Claim 42 and 44, Wittwer et al. teaches co-amplifying two or more separate regions of nucleic acid using at least two sets of PCR primers and at least two sets of FRET oligonucleotide pairs as probes to simultaneously genotype the separate regions by analyzing the melting temperature of the sets of FRET oligonucleotide pairs (Column 16 lines 11-20).

With regard to Claim 43, Wittwer et al. teaches the melting peaks of each set of probes must be distinguishable from the next set of probes (Column 15 lines 5-10). Wittwer et al. teaches to distinguish the melting point peaks of two sets of probes the probes are designed so the melting temperature of each set of probes is different from the melting temperature of the other set of probes (Column 15, lines 5-10). Wittwer et al. teaches that to differentiate amplification products the melting temperature can be separated by less than 2°C (column 16, lines 5-10).

Wittwer et al. however does not directly detecting the detectable signals of the signal probes as a function of temperature.

With regard to Claims 1, 10, 36 and 46-49, Marras et al. teaches a method of contact mediated quenching in oligonucleotide probes (abstract). Marras et al. teaches oligonucleotides are synthesized such that a quencher is at the end of on of the nucleotides and a signal is at the end of another oligonucleotide (p. 2 2nd column 1st paragraph). Such that like Wittwer et al., Marras et al. teaches the design of probes with either a quencher or a signal attached.

Marras et al. teaches that these probes can be measures such that changes in the intensity of florescence (e.g. the signal) is measured directly (p. 2 1st column 2nd full paragraph).

With regard to Claims 52 and 55, Marras et al. teaches first quencher probe and a second quencher probe which is nonfluroescent (p. 2 1st column last paragraph).

With regard to Claims 53-54 and 56, Marras et al. teaches that the signal can be detected both in FRET detection a non-FRET mechanism (p. 2 1st column last paragraph).

With regard to Claim 57, Marras et al. teaches a method wherein the detectable signal from the signal probe is measures (p. 2 1st column 2nd full paragraph).

With regard to Claims 58-59 and 61-62, Marras et al. teaches a method of measuring the detectable signal a decreasing and increasing temperatures and predetermined intervals (Figure 4).

Therefore it will be prima facie obvious to one of ordinary skill in the art at the time of filing to modify the teaching of Wittwer et al. method of detecting multiple signals based on melting temperature analysis to be measured such that the signal is measured directly as taught by Marras et al. with a reasonable expectation of success. The ordinary artisan would be motivated to measure directly the signal in a Quencher/Signal complex, because Marras et al. teaches that the direct measurement leads to the improvement of multiplexing because the signals are measured directly

rather than through detection of an alteration in the shape of the emission spectrum (p. 2 1st column 2nd full paragraph).

Response to Arguments

The reply traverses the rejection made of record of Wittwer et al., however, these arguments will be discussed below in view of the newly applied as necessitated by amendment rejection of Wittwer et al. in view of Marras et al. Response to arguments follows the summary of arguments made in the reply.

The reply asserts that Wittwer measures FRET and as such does not detect the direct detection of the signal probe (p. 15 last two paragraph).

This argument has been fully reviewed but has not been found persuasive.

As discussed in the rejection set forth above, though Wittwer et al. does not directly detect the signal probe, the method of Wittwer et al. provides all other claim steps and Marras has been used to supplement the teachings of Wittwer. The combination of Wittwer et al. in view of Marras et al. provides for the step of detection of the signal and motivation of why the ordinary artisan would be motivated to detect such a signal. The ordinary artisan would be motivated to measure directly the signal in a Quencher/Signal complex, because Marras et al. teaches that the direct measurement leads to the improvement of multiplexing because the signals are measured directly rather than through detection of an alteration in the shape of the emission spectrum (p. 2 1st column 2nd full paragraph). Therefore using the method steps of Marras et al would provide for an improved multiplexing method.

12. Claims 5 and 7-8 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wittwer et al. (US Patent 6,140,054 October 31, 2000) in view of Marras et al. (Nucleic acids Research 2002 Vol. 30 p. e122) as applied to Claims 1-4, 6, 9-10, 26-28, 31-33, 36-44, 46-59, and 61-62 and further in view of Caplin et al. (Biochemical 1999 No. 1 p. 5).

Wittwer et al. and Marras et al. teach a method of measuring the difference in a sample as a function of melting temperature by directly detecting the signal probe.

With regard to Claim 8, Wittwer et al. teaches to distinguish the melting point peaks of two sets of probes the probes are designed so the melting temperature of each set of probes is different from the melting temperature of the other set of probes (Column 15, lines 5-10). Wittwer et al. teaches that to differentiate amplification products the melting temperature can be separated by less than 2°C (column 16, lines 5-10).

Wittwer et al. and Marras et al., however, do not teach the melting temperature differences between the probes.

Caplin et al. teaches a method of direct mutation detection (abstract). With regard to Claims 5 and 7, Caplin et al. teaches for mutation detection, the best melting curves are obtained when the difference between the probes (quencher and signal) is between 5-10°C (p. 6 1st paragraph). With regard to Claim 8, Caplin teaches that for

detection the melting temperature of hybridization probes should be within 2°C (p. 6 1st paragraph).

Therefore it would have been prime facie obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Wittwer et al. and Marras et al. to use the melting temperature differences between probes as taught by Caplin et al. The ordinary artisan would have been motivated to modify the method of Wittwer et al. and Marras et al. because Caplin et al. teaches the best melting curves are obtained with the specific temperature difference of 5-10°C (p. 6 1st paragraph). The ordinary artisan would be motivated to create the second signal probe at least 7 degrees less than the first signal probe to ensure that the second set could be detected (5 degrees from the quencher and 2 degrees below the other hybridized probe).

Response to Arguments

The reply traverses the rejection. The reply asserts that Caplin et al. does not teach direct detection of the signal probe (p. 19 last two paragraphs).

This argument has been fully considered but has not been found persuasive.

Though neither Caplin et al. or Wittwer et al. teach the direct detection of the signal probe, the combination with Marras et al. teaches the limitation of the direct detection of the signal probe. The ordinary artisan would be motivated to measure directly the signal in a Quencher/Signal complex, because Marras et al. teaches that the direct measurement leads to the improvement of multiplexing because the signals are measured directly rather than through detection of an alteration in the shape of the

emission spectrum (p. 2 1st column 2nd full paragraph). Therefore using the method steps of Marras et al would provide for an improved multiplexing method.

Therefore the combination of Wittwer et al in view of Marras et al. and Caplin et al teaches all the limitation of the claims.

13. Claims 11-15 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wittwer et al. (US Patent 6,140,054 October 31, 2000) in view of Marras et al. (Nucleic acids Research 2002 Vol. 30 p. e122) as applied to Claims 1-4, 6, 9-10, 26-28, 31-33, 36-44, 46-59, and 61-62 and further in view of Tsourkas et al. (Nucleic acid research 2002 Vol. 30 p. 5168).

Wittwer et al. and Marras et al. teach a method of measuring the difference in a sample as a function of melting temperature by directly detecting the signal probe.

Wittwer et al. and Marras et al., however, do not teach the use of self-indicating signal probes such as hairpin probes.

Tsourkas et al. teaches a method of using methyl labeled molecular beacons to increase hybridization results (Abstract). With regard to Claims 11 and 12, Tsourkas teaches molecular beacons is a stem-loop confirmation (hairpin) that is quenched until it opens when hybridized to a target (2nd column 1st paragraph). With regard to Claims 13-15, Tsourkas et al. teaches that false-positive signals are fluorescence signals induced by the opening of molecular beacons due to nucleases (p. 5168 2nd column 2nd

paragraph). Tsourkas et al. teaches that nuclease degradation can be reduced by adding phosphorothioate, PNA, and 2'-O-methyl (p. 5168 2nd column 2nd paragraph). Tsourkas et al. teaches that false-positives can be further reduced by using two molecule beacons that bind to adjacent regions on a target molecule and generate positive signals via FRET (p. 5168 2nd column 2nd paragraph).

Therefore it would have been prime facie obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Wittwer et al. and Marras et al. to use hairpin probes as signal probes as taught by Tsourkas et al. The ordinary artisan would have been motivated to modify the method of Wittwer et al. and Marras et al. because Tsourkas et al. teaches tem-loop structure of a molecular beacon improves the specificity of target discrimination compared with linear probes (p. 5168 2nd column 1st paragraph). Tsourkas et al. teaches the competing reaction between hairpin formation and target hybridization increases the sensitivity of detecting a SNP between probe and target sequences and thus enables molecular beacons to differentiated between wild-type and mutants targets better than linear probes (p. 5168 2nd column 1st paragraph).

Response to Arguments

The reply traverses the rejection. The reply asserts that Tsourkas et al. does not teach direct detection of the signal probe (p. 16 4th paragraphs).

This argument has been fully considered but has not been found persuasive.

Though neither Tsourkas et al. or Wittwer et al. teach the direct detection of the signal probe, the combination with Marras et al. teaches the limitation of the direct

detection of the signal probe. The ordinary artisan would be motivated to measure directly the signal in a Quencher/Signal complex, because Marras et al. teaches that the direct measurement leads to the improvement of multiplexing because the signals are measured directly rather than through detection of an alteration in the shape of the emission spectrum (p. 2 1st column 2nd full paragraph). Therefore using the method steps of Marras et al would provide for an improved multiplexing method.

Therefore the combination of Wittwer et al in view of Marras et al. and Tsourkas et al. teaches all the limitation of the claims.

14. Claims 11-15 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wittwer et al. (US Patent 6,140,054 October 31, 2000) in view of Marras et al. (Nucleic acids Research 2002 Vol. 30 p. e122) as applied to Claims 1-4, 6, 9-10, 26-28, 31-33, 36-44, 46-59, and 61-62 and further in view of Sokol et al. (PNAS 1998 Vol 95 p. 11538).

Wittwer et al. and Marras et al. teach a method of measuring the difference in a sample as a function of melting temperature by directly detecting the signal probe.

Wittwer et al. and Marras et al., however, do not teach the use of self-indicating signal probes such as hairpin probes.

Sokol et al. teaches a method of real time detection of DNA and RNA hybridization in living cells (Abstract). With regard to Claims 11-13, Sokol et al. teaches the use of Molecular beacons (hairpin probes) as the reporter (signal) oligonucleotide (abstract).

The instant specification defines "self-indicating probes" as a signal probe that produces little or no detectable signal when free in solution (p. 7-8 paragraph 80). Sokol et al. teaches molecular beacons when in the stem loop confirmation (not hybridized so therefore in solution) produce no signal emission (abstract). With regard to Claim 13, Sokol et al. teaches molecular beacons provide greater nuclease resistance (p. 11541 last sentence). With regard to Claims 14-15, Sokol et al. teaches using DNA molecular beacons (p. 11538 last paragraph).

Therefore it would have been prime facie obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Wittwer et al. and Marras et al. to use molecular beacons as signal probes as taught by Sokol et al. The ordinary artisan would have been motivated to modify the method of Wittwer et al. and Marras et al. because Sokol et al. teaches a potential problem with FRET detection is that the loss of fluorescence might not mean conclusively that hybridization has taken place therefore the use of molecular beacons allows for direct demonstration that duplex formation took place and FRET was suppressed (p. 11538 2nd column first full paragraph).

Response to Arguments

The reply traverses the rejection. The reply asserts that Sokol et al. does not teach direct detection of the signal probe (p. 17 1st paragraph).

This argument has been fully considered but has not been found persuasive.

Though neither Sokol et al. or Wittwer et al. teach the direct detection of the signal probe, the combination with Marras et al. teaches the limitation of the direct detection of the signal probe. The ordinary artisan would be motivated to measure directly the signal in a Quencher/Signal complex, because Marras et al. teaches that the direct measurement leads to the improvement of multiplexing because the signals are measured directly rather than through detection of an alteration in the shape of the emission spectrum (p. 2 1st column 2nd full paragraph). Therefore using the method steps of Marras et al would provide for an improved multiplexing method.

Therefore the combination of Wittwer et al in view of Marras et al. and Sokol et al teaches all the limitation of the claims.

15. Claims 11, 16-20, and 38 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wittwer et al. (US Patent 6,140,054 October 31, 2000) in view of Marras et al. (Nucleic acids Research 2002 Vol. 30 p. e122) as applied to Claims 1-4, 6, 9-10, 26-28, 31-33, 36-44, 46-59, and 61-62 and further in view of Kubista et al (US Patent 6329144 December 11, 2001).

Wittwer et al. and Marras et al. teach a method of measuring the difference in a sample as a function of melting temperature by directly detecting the signal probe.

Wittwer et al. and Marras et al., however, do not teach use of self-indicating signal probes.

Kubista et al. teaches a method of using a probe for the detecting nucleic acids having a particular sequence (Abstract). With regard to Claims 11, 16, and 18-20, Kubista et al. teaches the use of PNA based probes. The instant specification defines "self-indicating probes" as a signal probe that produces little or no detectable signal when free in solution (p. 7-8 paragraph 80). Kubista et al. teaches a method that has minimal luminescence free in solution and strong luminescence bound to nucleic acids (Column 6 lines 43-45). With regard to Claim 17, Kubista et al. teaches that PNA based probes are resistant to nucleases (Column 9, line 6). With regard to Claim 38, Kubista et al. teaches the PNA forms a more rigid duplex with single stranded than double stranded nucleic acids which more effectively restricts the internal motion in the bound reporter and increases fluorescence signal (Column 10 lines 63-65).

Therefore it would have been prime facie obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Wittwer et al. and Marras et al. to use PNA probes as taught by Kubista et al. The ordinary artisan would have been motivated to modify the method of Wittwer et al. and Marras et al. because Kubista et al. teaches PNA based probes obtain a higher signal than nucleic acid based probes when hybridized to the target strand (Column 18, lines 56-57). The ordinary artisan would use a method, which had the highest measurable signal in order to be able to determine signal versus background signal more efficiently.

Response to Arguments

The reply traverses the rejection. The reply asserts that Kubista et al. does not teach direct detection of the signal probe (p. 17 5th paragraph).

This argument has been fully considered but has not been found persuasive.

Though neither Kubista et al. or Wittwer et al. teach the direct detection of the signal probe, the combination with Marras et al. teaches the limitation of the direct detection of the signal probe. The ordinary artisan would be motivated to measure directly the signal in a Quencher/Signal complex, because Marras et al. teaches that the direct measurement leads to the improvement of multiplexing because the signals are measured directly rather than through detection of an alteration in the shape of the emission spectrum (p. 2 1st column 2nd full paragraph). Therefore using the method steps of Marras et al would provide for an improved multiplexing method.

Therefore the combination of Wittwer et al in view of Marras et al. and Kubista et al teaches all the limitation of the claims.

16. Claims 11 and 21-23 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wittwer et al. (US Patent 6,140,054 October 31, 2000) in view of Marras et al. (Nucleic acids Research 2002 Vol. 30 p. e122) as applied to Claims 1-4, 6, 9-10, 26-28, 31-33, 36-44, 46-59, and 61-62 and further in view of Tyagi et al (US Patent 6277607 August 21, 2001).

Wittwer et al. and Marras et al. teach a method of measuring the difference in a sample as a function of melting temperature by directly detecting the signal probe.

Wittwer et al. and Marras et al., however, do not teach using an intercalating dye.

Tyagi et al. teaches a method of nucleic acid amplification in which primers are in hairpin structures (abstract). With regard to Claim 11, Tyagi et al. teaches the use of hairpin primers, which can monitor the amplification reactions by florescence (Column 6, lines 52-53). With regard to Claims 21-23, Tyagi et al. teaches that the assays can be detected using intercalating dyes (Column 4, lines 6-10). Tyagi et al. teaches that one type of intercalating dye that can be used is SYBR Green (Column 11, lines 39-41).

Therefore it would have been prime facie obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Wittwer et al. and Marras et al. to use hairpin probes and intercalating dye as taught by Tyagi et al. The ordinary artisan would have been motivated to modify the method of Wittwer et al. and Marras et al. because Tyagi et al. teaches the use of hairpin probes reduces the probability of primer dimers, allows for real-time detection of the amplification product for accurate quantification of the initial number of target sequences in a sample (Column 7, lines 1-15). Tyagi et al. teaches that the use of an intercalating dye allows for the monitoring of each reaction (Column 11, lines 35-40).

Response to Arguments

The reply traverses the rejection. The reply asserts that Tyagi et al. does not teach direct detection of the signal probe (p. 18 2nd paragraph).

This argument has been fully considered but has not been found persuasive.

Though neither Tyagi et al. or Wittwer et al. teach the direct detection of the signal probe, the combination with Marras et al. teaches the limitation of the direct

detection of the signal probe. The ordinary artisan would be motivated to measure directly the signal in a Quencher/Signal complex, because Marras et al. teaches that the direct measurement leads to the improvement of multiplexing because the signals are measured directly rather than through detection of an alteration in the shape of the emission spectrum (p. 2 1st column 2nd full paragraph). Therefore using the method steps of Marras et al would provide for an improved multiplexing method.

Therefore the combination of Wittwer et al in view of Marras et al. and Tyagi et al teaches all the limitation of the claims.

17. Claims 24-25 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wittwer et al. (US Patent 6,140,054 October 31, 2000) in view of Marras et al. (Nucleic acids Research 2002 Vol. 30 p. e122) as applied to Claims 1-4, 6, 9-10, 26-28, 31-33, 36-44, 46-59, and 61-62 and further in view of Singer et al. (US Patent 6,323,337 November 27, 2001).

Wittwer et al. and Marras et al. teach a method of measuring the difference in a sample as a function of melting temperature by directly detecting the signal probe. Wittwer et al. and Marras et al., however, do not teach use a fluorescent minor groove binding dye.

Singer et al. teaches a method to label oligonucleotides (abstract). With regard to Claims 24 and 25, Singer et al. teaches the use of nucleic acid stain such as Hoechst 33342, Hoechst 34580, and DAPI (Column 14 lines 19-25).

Therefore it would have been prime facie obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Wittwer et al. and Marras et al. to use intercalating dye as taught by Singer et al. The ordinary artisan would have been motivated to modify the method of Wittwer et al. and Marras et al. because Singer et al. teaches dyes, which are useful in the combination of quenching oligonucleotides to minimize the fluorescent signal from selected oligonucleotides (Column 16, lines19-22).

Response to Arguments

The reply traverses the rejection. The reply asserts that Singer et al. does not teach direct detection of the signal probe (p. 18 2nd to last paragraph).

This argument has been fully considered but has not been found persuasive.

Though neither Singer et al. or Wittwer et al. teach the direct detection of the signal probe, the combination with Marras et al. teaches the limitation of the direct detection of the signal probe. The ordinary artisan would be motivated to measure directly the signal in a Quencher/Signal complex, because Marras et al. teaches that the direct measurement leads to the improvement of multiplexing because the signals are measured directly rather than through detection of an alteration in the shape of the

emission spectrum (p. 2 1st column 2nd full paragraph). Therefore using the method steps of Marras et al would provide for an improved multiplexing method.

Therefore the combination of Wittwer et al in view of Marras et al. and Singer et al teaches all the limitation of the claims.

18. Claim 45 is rejected under 35 U.S.C. 103(a) as being unpatentable over Wittwer et al. (US Patent 6,140,054 October 31, 2000) in view of Marras et al. (Nucleic acids Research 2002 Vol. 30 p. e122) as applied to Claims 1-4, 6, 9-10, 26-28, 31-33, 36-44, 46-59, and 61-62 and further in view of Schalasta et al. (Infection 2000 Vol 28 p. 85).

Wittwer et al. and Marras et al. teach a method of measuring the difference in a sample as a function of melting temperature by directly detecting the signal probe.

Wittwer et al. and Marras et al., however, do not teach using the method for virus genotyping.

With regard to Claim 45, Schalasta et al. teaches a method of genotyping type-specific HPV Type 1 and Type 2 using fluorescence Melting Curve analysis (Abstract).

Therefore it would have been prime facie obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Wittwer et al. and Marras et al. to use in genotyping viruses as taught by Schalasta et al. The ordinary artisan would have been motivated to use the method of Wittwer et al. and Marras et al.

because Schalasta et al. teaches the use of FRET based melting curve analysis provides a rapid diagnosis, high sensitivity, and specificity (abstract).

Response to Arguments

The reply traverses the rejection. The reply asserts that Schalasta et al. does not teach direct detection of the signal probe (p. 19 3rd paragraph).

This argument has been fully considered but has not been found persuasive.

Though neither Schalasta et al. or Wittwer et al. teach the direct detection of the signal probe, the combination with Marras et al. teaches the limitation of the direct detection of the signal probe. The ordinary artisan would be motivated to measure directly the signal in a Quencher/Signal complex, because Marras et al. teaches that the direct measurement leads to the improvement of multiplexing because the signals are measured directly rather than through detection of an alteration in the shape of the emission spectrum (p. 2 1st column 2nd full paragraph). Therefore using the method steps of Marras et al would provide for an improved multiplexing method.

Therefore the combination of Wittwer et al in view of Marras et al. and Schalasta et al teaches all the limitation of the claims.

19. Claims 29 and 34 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wittwer et al. (US Patent 6,140,054 October 31, 2000) in view of Marras et al. (Nucleic acids Research 2002 Vol. 30 p. e122) as applied to Claims 1-4, 6, 9-10, 26-28,

31-33, 36-44, 46-59, and 61-62 and further in view of Elenitoba-Johnson (US Patent 6346386 February 12, 2002).

Wittwer et al. and Marras et al. teach a method of measuring the difference in a sample as a function of melting temperature by directly detecting the signal probe.

Wittwer et al. and Marras et al., however, do not teach temperature rate range.

Elenitoba-Johnson teaches a method for determining alterations in a nucleic acid using a fluorescent label and melting profiles (Abstract). With regard to Claims 29 and 34, Elenitoba-Johnson teaches a method of performing RT PCR in which the temperature of the sample is raised at a rate slow enough to distinguish between the melting points of the wild type and the fragment of interest (Column 7 lines 5-10). Elenitoba-Johnson teaches a ramping rate of between 0.1 °C/sec to 0.01°C/sec (6°C/min to .6 °C/min) (Column 7, lines 11-20). Elenitoba-Johnson teaches a PCR amplification method in which the probes and target are increased above the melting point of the probes to below the melting point of the probes (Column 10 Example 3).

Therefore it would have been prime facie obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Wittwer et al. and Marras et al. to use the ramping speed as taught by Elenitoba-Johnson et al. The ordinary artisan would have been motivated to modify the method of Wittwer et al. and Marras et al. because Elenitoba-Johnson et al. teaches a ramping speed in which it is slow enough to distinguish between the melting temperatures of two sequences (Column 7, lines 1-5).

Response to Arguments

The reply traverses the rejection. The reply asserts that Elenitoba-Johnson et al. does not teach direct detection of the signal probe (p. 21 1st paragraph).

This argument has been fully considered but has not been found persuasive.

Though neither Elenitoba-Johnson et al. or Wittwer et al. teach the direct detection of the signal probe, the combination with Marras et al. teaches the limitation of the direct detection of the signal probe. The ordinary artisan would be motivated to measure directly the signal in a Quencher/Signal complex, because Marras et al. teaches that the direct measurement leads to the improvement of multiplexing because the signals are measured directly rather than through detection of an alteration in the shape of the emission spectrum (p. 2 1st column 2nd full paragraph). Therefore using the method steps of Marras et al would provide for an improved multiplexing method.

Therefore the combination of Wittwer et al in view of Marras et al. and Elenitoba-Johnson et al teaches all the limitation of the claims.

20. Claims 30, 35, 60, and 63 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wittwer et al. (US Patent 6,140,054 October 31, 2000) in view of Marras et al. (Nucleic acids Research 2002 Vol. 30 p. e122) as applied to Claims 1-4, 6, 9-10, 26-28, 31-33, 36-44, 46-59, and 61-62 and further in view of Witter et al. (named Witter B) (US Patent 6,245,514 June 2001) .

Wittwer et al. and Marras et al. teach a method of measuring the difference in a sample as a function of melting temperature by directly detecting the signal probe.

Wittwer et al. and Marras et al., however, do not teach the rate of monitoring the detectable signal.

With regard to Claims 30, 35, 60, and 63, Wittwer B teaches a method of measuring FRET pairs for detecting the presences of a target analyte (Abstract). Wittwer B teaches measuring fluorescent continuously every 200 msec as a function of temperature (Column 35 lines 29-33).

Therefore it would have been prime facie obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Wittwer et al. and Marras et al. to use the monitoring rate as taught by Wittwer B. The ordinary artisan would have been motivated to modify the method of Wittwer et al. and Marras et al. because Wittwer B teaches a monitoring rate in which data can be obtained by measuring fluorescence at each temperature (Column 37, lines 1-5). The ordinary artisan would want to modify the method in order to gain as much information as possible during the monitoring phase in order to make a more precise fluorescence vs. melting temperature curve.

Response to Arguments

The reply traverses the rejection. The reply asserts that Witter et al. (named Witter B) does not teach direct detection of the signal probe (p. 21 2nd to last paragraph).

This argument has been fully considered but has not been found persuasive.

Though neither Witter et al. (named Witter B) or Wittwer et al. teach the direct detection of the signal probe, the combination with Marras et al. teaches the limitation of the direct detection of the signal probe. The ordinary artisan would be motivated to measure directly the signal in a Quencher/Signal complex, because Marras et al. teaches that the direct measurement leads to the improvement of multiplexing because the signals are measured directly rather than through detection of an alteration in the shape of the emission spectrum (p. 2 1st column 2nd full paragraph). Therefore using the method steps of Marras et al would provide for an improved multiplexing method.

Therefore the combination of Wittwer et al in view of Marras et al. and Witter et al. (named Witter B) teaches all the limitation of the claims.

Conclusion

21. No claims are allowed.

22. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Katherine Salmon whose telephone number is (571) 272-3316. The examiner can normally be reached on Monday-Friday 8AM-430PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ram Shukla can be reached on (571) 272-0735. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

/Katherine Salmon/
Examiner, Art Unit 1634

/Ram R. Shukla/

Supervisory Patent Examiner, Art Unit 1634